

Transcriptome Analysis of Psoriasis in a Large Case–Control Sample: RNA-Seq Provides Insights into Disease Mechanisms

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To increase our understanding of psoriasis, we used high-throughput complementary DNA sequencing (RNA-seq) to assay the transcriptomes of lesional psoriatic and normal skin. We sequenced polyadenylated RNA-derived complementary DNAs from 92 psoriatic and 82 normal punch biopsies, generating an average of ~38 million single-end 80-bp reads per sample. Comparison of 42 samples examined by both RNA-seq and microarray revealed marked differences in sensitivity, with transcripts identified only by RNA-seq having much lower expression than those also identified by microarray. RNA-seq identified many more differentially expressed transcripts enriched in immune system processes. Weighted gene coexpression network analysis (WGCNA) revealed multiple modules of coordinately expressed epidermal differentiation genes, overlapping significantly with genes regulated by the long noncoding RNA *TINCR*, its target gene, *stau1* (*STA1*), the p63 target gene *ZNF750*, and its target *KLF4*. Other coordinately expressed modules were enriched for lymphoid and/or myeloid signature transcripts and genes induced by IL-17 in keratinocytes. Dermally expressed genes were significantly downregulated in psoriatic biopsies, most likely because of expansion of the epidermal compartment. These results show the power of WGCNA to elucidate gene regulatory circuits in psoriasis, and emphasize the influence of tissue architecture in both differential expression and coexpression analysis.

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INTRODUCTION

Psoriasis is a chronic inflammatory disease of the skin and joints, affecting ~0.2–2% of the world's population (Nestle *et al.*, 2009; Gudjonsson and Elder, 2012). Microarray-based studies of the psoriatic transcriptome have revealed a large number of differentially expressed genes (DEGs) in lesional skin (Bowcock *et al.*, 2001; Zhou *et al.*, 2003; Reischl *et al.*,

2007; Yao *et al.*, 2008; Zaba *et al.*, 2009; Gudjonsson *et al.*, 2010; Suarez-Farinas *et al.*, 2010), both in case–control comparisons and in lesional and normal skin from the same individual. Moreover, one study of nonlesional psoriatic versus normal skin identified over 200 DEGs (Gudjonsson *et al.*, 2009). Recently, high-throughput complementary DNA sequencing (RNA-seq) has emerged as a powerful alternative to microarrays (Wang *et al.*, 2009; Metzker, 2010; Garber *et al.*, 2011; Liu *et al.*, 2011; Ozsolak and Milos, 2011; Roy *et al.*, 2011) that provides increased accuracy and precision (Roy *et al.*, 2011), identifies unmapped transcripts and alternative splicing, and markedly can increase the dynamic range of detection (Wang *et al.*, 2009; Liu *et al.*, 2011). To date, there has been only one study using RNA-seq to analyze the coding psoriatic transcriptome (Jabbari *et al.*, 2012), examining three pairs of lesional and normal skin samples from psoriatic individuals, and one other study limited to the microRNA genome (Joyce *et al.*, 2011).

In this paper, we report findings from a large-scale RNA-seq analysis of skin biopsies from 174 individuals. A subset of these samples has been studied previously using microarrays (Gudjonsson *et al.*, 2009, 2010), allowing for a robust comparison of the two technologies. In addition, the large sample size we consider permits new modes of data analysis, such as gene coexpression network analysis (Barabasi and

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Abbreviations: DEG, differentially expressed gene; FC, fold-change; RNA-seq, high-throughput complementary DNA sequencing; WGCNA, weighted gene coexpression network analysis

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Oltvai, 2004; Zhang and Horvath, 2005; Langfelder and Horvath, 2007, 2008; Yip and Horvath, 2007; Horvath and Dong, 2008; De Las Rivas and Fontanillo, 2010). Our results show the value of RNA-seq in capturing the full dynamic range of the psoriatic transcriptome, identify key transcription factors involved in psoriasis, and reveal gene coexpression networks illuminating the processes of keratinocyte differentiation, lipid biosynthesis, and the inflammatory interplay between myeloid cells, T cells, and keratinocytes. We also show that expansion of the epidermal compartment provides an architectural explanation for the apparent downregulation of dermally derived transcripts in psoriatic lesions.

RESULTS

Comparison of RNA-seq and microarray results

Using the Illumina GAII platform, we sequenced libraries constructed from complementary DNA prepared from polyadenylated RNA extracted from skin biopsies donated by 92 psoriatic patients and 82 normal individuals. From reads averaging 80bp in length, we generated on average 38 ± 10 (mean \pm SD) million reads per sample, yielding approximately 3 gigabases of complementary DNA sequence per sample. We aligned the reads to the NCBI build 37 reference genome using TopHat (Garber *et al.*, 2011), yielding an average mapping rate of $80 \pm 6\%$ (mean \pm SD). After performing quality control (see Materials and Methods), gene expression levels, represented as reads per kilobase per million mapped reads (RPKM) (Mortazavi *et al.*, 2008), were obtained for 21,099 genes annotated in the RefSeq database (Pruitt *et al.*, 2007). We identified 3,577 DEGs between lesional and normal skin ($|\log_2 FC| \geq 1$ and $P < 1 \times 10^{-6}$, corresponding to family-wise error rate < 0.025), of which 1,049 were upregulated and 2,528 were downregulated in psoriasis (the full list of DEGs is provided in Supplementary Table S1 online). We then performed analysis to investigate the potential splicing events based on differential usage of exons (Anders *et al.*, 2012). We identified 343 exons (from 292 genes) with differential usage between the psoriatic and normal skin conditions. Notably, only 95 of the genes were differentially expressed based on the gene-level analysis (Supplementary Table S1 online).

In previous studies (Gudjonsson *et al.*, 2009, 2010), we measured gene expression using Affymetrix HU133 Plus 2.0 microarrays on 122 skin samples (58 lesional and 64 normal). Among these, 42 samples overlap with the present experiment (20 lesional and 22 normal). We first examined the expression levels for the 42 samples assayed on both platforms. In general, RNA-seq and microarray measurements were consistent for intermediate- and high-abundance transcripts, whereas discrepancies become noticeable for low-abundance transcripts in both cases and controls (Supplementary Figure S1 online). We then compared each gene's fold-change (FC) estimates derived using microarrays with those obtained by RNA-seq (Supplementary Figure S2a online). For medium- and high-abundance transcripts, FC estimates were relatively consistent between the two platforms (Supplementary Figure S2c and d online). In contrast, for low-abundance transcripts (Supplementary Figure S2b online), RNA-seq had a wider

range of FC estimates, which is in agreement with other studies (Marioni *et al.*, 2008; Bradford *et al.*, 2010).

We next compared our set of DEGs with our full microarray data set (Gudjonsson *et al.*, 2010). Over 80% (i.e., 794/987) of the genes identified in the microarray analysis were also flagged by RNA-seq, but only 22% (794/3,577) of the genes identified by RNA-seq were flagged by microarray analysis (Supplementary Figure S3 online). Again, DEGs detected only by RNA-seq tended to be low-abundance transcripts (Supplementary Figure S4 online, panels a and b), whereas those identified by both platforms (Supplementary Figure S4 online, panels c and d) or only using microarrays (Supplementary Figure S4 online, panels e and f) tended to be intermediate- and high-abundance transcripts. We observed very similar patterns when we restricted the analysis to only those genes and samples that overlapped between the RNA-seq and microarray experiments (Supplementary Figure S5 online). We did not observe any difference in the distributions of gene length, number of exons, and number of transcripts in genes identified as DEGs only by RNA-seq, only by microarrays, or by both platforms (Supplementary Figure S6 online). Using the same statistical criteria that we have used in this analysis, we reprocessed available microarray data in order to compare the number and percentage of the overlap of DEGs between our data sets and other independent data sets (Zaba *et al.*, 2009; Suarez-Farinas *et al.*, 2012). These results are presented in Supplementary Table S2 online.

Analysis of DEGs

We performed pathway analyses to identify biological functions/pathways enriched among the DEGs. The most significantly enriched functions among the upregulated genes included "inflammatory responses" ($P = 6 \times 10^{-31}$), "cytokine-receptor interactions" ($P = 6 \times 10^{-27}$), "cell division" ($P = 2 \times 10^{-15}$), and "keratinization" ($P = 4 \times 10^{-14}$). Using the cytokine-cytokine receptor interaction pathway as an example, 14 of the 17 genes having the highest differences in expression between psoriatic and normal skin were not identified as DEGs in a recent microarray-based study of psoriatic skin (Gudjonsson *et al.*, 2010; Supplementary Table S3 online). We performed quantitative real-time reverse-transcriptase-PCR experiments on 8 out of the 17 genes listed in Supplementary Table S3 online, and validated that their expression levels are all significantly differentially expressed (Supplementary Table S3 online). The most significantly enriched functions among the downregulated genes included "calcium ion binding" ($P = 4 \times 10^{-21}$), "homophilic cell adhesion" ($P = 5 \times 10^{-20}$), and "muscle contraction" ($P = 1 \times 10^{-17}$). Subsequent analysis revealed that many of these genes were of dermal origin and systematically underexpressed owing to expansion of the epidermal compartment in psoriatic lesions (see below). However, the 16 DEGs showing enrichment for "keratinization" that were detected only by RNA-seq comprised a mixture of upregulated and downregulated genes, and only 4 of which were represented on the HU133 Plus 2.0 microarray platform. Supplementary Table S4 online shows the full list of significantly enriched functions and the genes contributing to each.

DEGs attributable to Th17 function (Ouyang *et al.*, 2008) were prominent in our sample, including *IL-12B*, *IL-17A*, *IL-17F*, *IL-21*, *IL-22*, *IL-26*, *IL-21R*, and *IL-23R*. With the exception of *IL-26* and *IL-21R*, increased expression of these genes has previously been described in lesional psoriasis (Teunissen *et al.*, 1998; Lee *et al.*, 2004; Caruso *et al.*, 2009). Although their expression in psoriatic skin was quite low, their expression was often undetectable in normal skin (Supplementary Table S2 online). Other notable immune-related genes that are significantly upregulated included IFN- γ (*IFNG*, >1000-fold), IFN- ϵ (*IFNE*, >1000-fold), nitric oxide synthase (*NOS2*, 50-fold), IL-6 (*IL-6*, 4.3-fold), and IL-24 (*IL-24*, 4-fold); IL-34 is significantly downregulated (IL-34, 7-fold suppressed).

To examine transcriptional mechanisms underlying the differential expression of genes identified by RNA-seq, we performed a transcription factor analysis using the Ingenuity Pathway Analysis software. The results revealed significant enrichment for the targets of 61 transcription factors (Supplementary Table S5 online). Of these, 15 were predicted to be in an activated state in psoriatic skin, 12 were predicted to be in an inhibited state, and the remaining 34 yielded no prediction regarding activation. Among those predicted to be activated, the most significant ($P < 1 \times 10^{-10}$) enrichment involved targets of the proinflammatory transcription factors signal transducer and activator of transcription 3 (STAT3), NF- κ B, CCAAT/enhancer-binding protein beta, and STAT1, whereas peroxisome proliferator-activated receptor- γ (PPAR- γ) and forkhead box protein A2 were predicted to be inhibited. PPAR- γ is a major regulator of genes involved in lipid metabolism, water transport, and cornified envelope formation during keratinocyte differentiation (Jiang *et al.*, 2011). Similar to PPAR- γ , forkhead box protein A2 regulates networks of genes controlling complex metabolic functions (Soccio *et al.*, 2011) and shares many target genes with PPAR- γ (Supplementary Table S5 online).

Gene coexpression network analysis

We grouped transcripts with correlated expression levels into gene coexpression modules using the weighted gene coexpression network analysis (WGCNA) approach, which maximizes the network's scale-free properties (Zhang and Horvath, 2005; Yip and Horvath, 2007; Horvath and Dong, 2008; Langfelder and Horvath, 2008). WGCNA identified 18 gene modules in normal skin and 27 in lesional psoriatic skin. Figure 1 shows the average reads per kilobase per million mapped reads, average Spearman correlations, and the proportions of significantly upregulated and downregulated genes in each of the modules. We then tested each module for enrichment of specific biological functions or pathways (Supplementary Table S6 online). Cluster annotations and FC values for each gene are provided in Supplementary Table S7 online.

Among the normal skin coexpression clusters, modules N10 and N15 had the highest proportion of upregulated genes (each over 12%), and both were enriched for genes involved in keratinization ($P = 2 \times 10^{-10}$) or located in the epidermal differentiation complex ($P = 2 \times 10^{-10}$; Supplementary

Table S6 online). Among modules identified in psoriatic skin, modules P20 and P26 had the highest proportion of upregulated genes (each over 70%). Module P20 was enriched for genes involved in inflammatory and immune responses ($P = 1 \times 10^{-19}$ and 6×10^{-18} , respectively). Over 80% of module P20 genes were upregulated in lesional skin, averaging ~ 9 -fold difference in expression. Moreover, the average Spearman correlations of expression levels for genes in module P20 was much higher for lesional skin ($r = 0.45$) than for normal skin ($r = 0.07$, Figure 1, Supplementary Table S6 online). Module P26 was significantly enriched for genes involved in keratinization ($P = 3 \times 10^{-20}$) and located in the epidermal differentiation complex ($P = 7 \times 10^{-19}$). Module P23 was not characterized by strong differential expression, but, similar to modules N10, N15, and P26, it was enriched for genes found in the epidermal differentiation complex ($P = 7 \times 10^{-25}$) and/or involved in keratinization ($P = 9 \times 10^{-25}$). Consistent with the functional annotation results, modules N10, N15, P23, and P26 are all significantly enriched with the top signature genes of keratinocytes and epidermis (Figure 2). As shown in Figure 3, these four modules also have the highest proportions of overlap with transcripts identified in previous studies as being expressed in the granular layer of the epidermis (Toulza *et al.*, 2007; Mattiuzzo *et al.*, 2011), and with genes regulated by the long noncoding RNA *TINCR* and its target gene *staufen-1* (*STAU1*), which have been shown to regulate terminal differentiation of the epidermis (Kretz *et al.*, 2012), as well as the p63 target gene *ZNF750* and its target *KLF4* (Sen *et al.*, 2012; Figure 3).

Normal skin-derived modules N6 and N18 were significantly enriched for dermal tissue signature transcripts (Figure 2) related to extracellular space, as well as collagen and glycosaminoglycan binding (Supplementary Table S6 online). Both of these modules had high proportions of downregulated genes (Figure 1 and Supplementary Table S6 online). The apparent under-representation of dermis-derived transcripts in psoriasis lesions was further assessed with the aid of an existing data set derived from laser-capture microdissection of lesional versus nonlesional psoriatic skin (Mitsui *et al.*, 2012). Using this data set, we identified two sets of transcripts that (a) were specific for either the epidermis or dermis (i.e., expression of genes are significantly elevated as compared with other 23 cell types, see Supplementary Information online for details), and (b) did not vary substantially in their expression in lesional versus nonlesional psoriatic skin based on the laser-capture microdissection experiment. We then calculated the FC values (psoriasis vs. normal) for each of these transcripts in our RNA-seq data set. As shown in Supplementary Figure S7 online, the FC distribution of epidermis-specific transcripts was centered on unity ($\log_2 \text{FC} = 0$), as expected. However, the $\log_2 \text{FC}$ value of the dermis-specific transcripts was centered around -1 . This significant under-representation of dermal transcripts (Wilcoxon rank-sum test $P < 2 \times 10^{-16}$ when comparing dermis-specific genes' FCs with those from epidermis-specific genes) can be explained by the marked expansion of the epidermis that is characteristic of psoriatic lesions (Supplementary Figure S7e online).

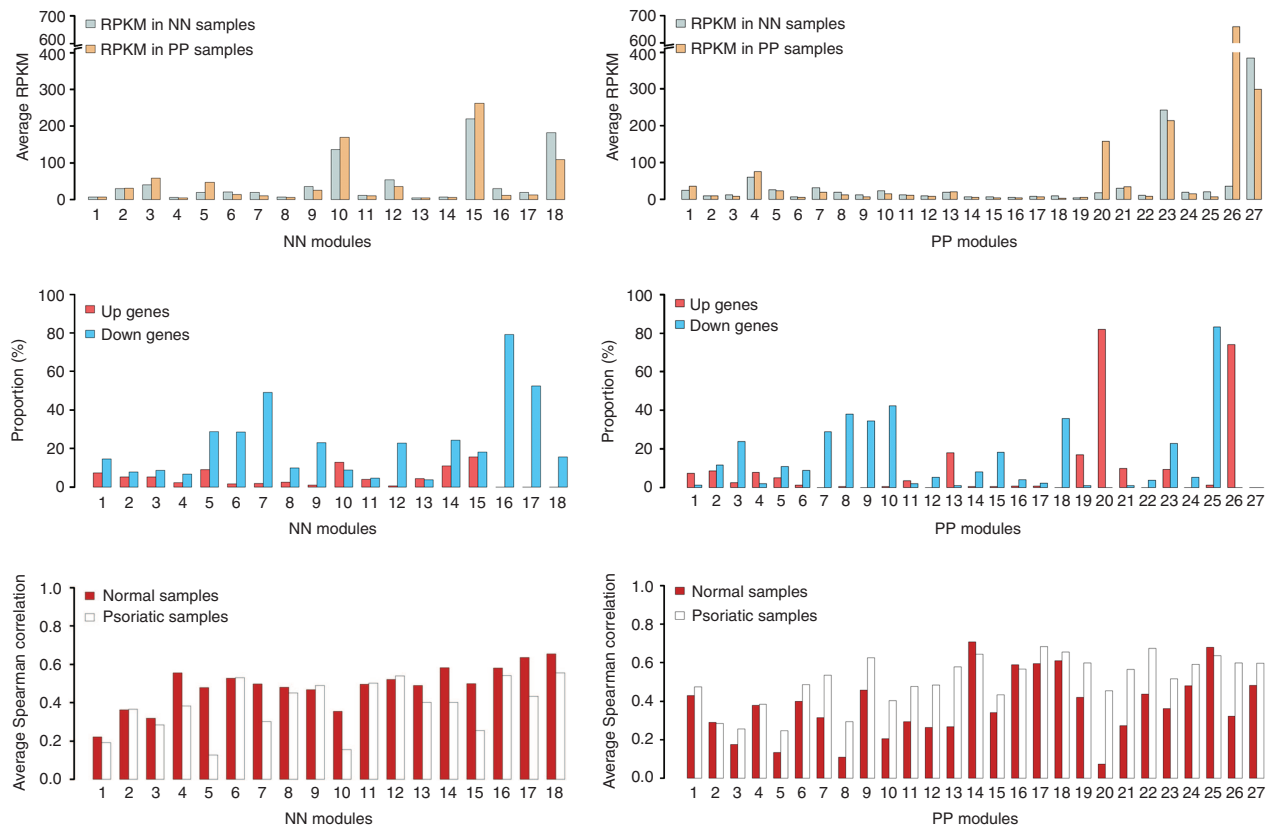


Figure 1. Averaged reads per kilobase per million mapped reads (RPKM) and proportion of DEGs for each module. Averaged RPKM among the genes (top panel), proportions of upregulated (Up) and downregulated (Down) genes (middle panel), and the average Spearman's correlation (bottom panel) for the coexpression gene modules constructed from the normal (normal; left panel) and psoriatic (psoriatic; right panel) skin samples, respectively.

The coexpressed gene modules with the most significantly enriched functional annotations were modules N14 and P18 (Supplementary Table S6 online), both of which were enriched with the function “keratin filament” ($P = 3 \times 10^{-113}$ for N14; $P = 8 \times 10^{-114}$ for P18). Indeed, 99 genes were common to both of these modules, and the majority (76/99) of them are hair keratins (Schweizer *et al.*, 2007) or keratin-associated proteins found mainly in hair follicles (Rogers *et al.*, 2006). Other hair follicle-specific genes mapped to these clusters include *S100A3* (Kizawa *et al.*, 2011), *DSG4* (Bazzi *et al.*, 2006), *PADI3* (Dong *et al.*, 2006), and trichohyalin (*TCHH*) (Steinert *et al.*, 2003). Moreover, both modules showed significant ($P < 1 \times 10^{-4}$) overlap (32% for N14; 26% for P18) with genes identified as downregulated in a paired microarray comparison of bald versus normal human scalp (Garza *et al.*, 2012). Moreover, the 25 most highly downregulated genes in that study included 18 of the same hair-specific keratins observed in clusters N14 and P18, including 9 hair keratins, 6 keratin-associated proteins, *S100A3*, and *TCHH*, all of which were downregulated by 4-fold or more in bald scalp.

The sebaceous gland and the arrector pili muscle are anatomically joined to the hair follicle to form the pilosebaceous unit (Lever and Schaumburg-Lever, 1990; Fujiwara *et al.*, 2011). Consistent with this, module N16 was significantly enriched for transcripts engaged in muscle contraction (Supplementary Table S6 online), and module N17 was

significantly enriched for signature genes expressed in adipose tissue (fat) but not in the dermis (Figure 2). Module N17 was also significantly enriched for PPAR signaling transcripts ($P = 1 \times 10^{-11}$) (Supplementary Table S6 online), which would be in keeping with the major role of PPAR- γ in adipocyte differentiation (Yessoufou and Wahli, 2010).

Three psoriatic modules (P13, P19, and P21) were significantly enriched ($P < 1 \times 10^{-18}$) for immune-related functions, had high proportions of upregulated genes, and exhibited higher Spearman's correlations in expression in lesional than in normal skin (Supplementary Table S6 online). They also contained the highest proportions of signature genes expressed in lymphocytes and/or myeloid cells (Figure 2). As shown in Figure 2, modules P13 and P19 were significantly enriched for the signatures of several types of lymphocytes, as well as dendritic cells, whereas module P21 manifested significant overlap for myeloid but not lymphoid signature genes.

In an effort to determine to what extent specific cytokines present in psoriatic lesions might influence the behavior of lesional keratinocytes, we used the signature gene enrichment approach to query coexpression modules expressed by keratinocytes and/or epidermis for genes distinctively expressed by cultured human keratinocytes treated with various cytokines (Swindell *et al.*, 2012, 2013). Interestingly, three (N15, P20, and P26) out of the five modules manifesting a strong keratinocyte gene expression signature (N10, N15, P20, P23,

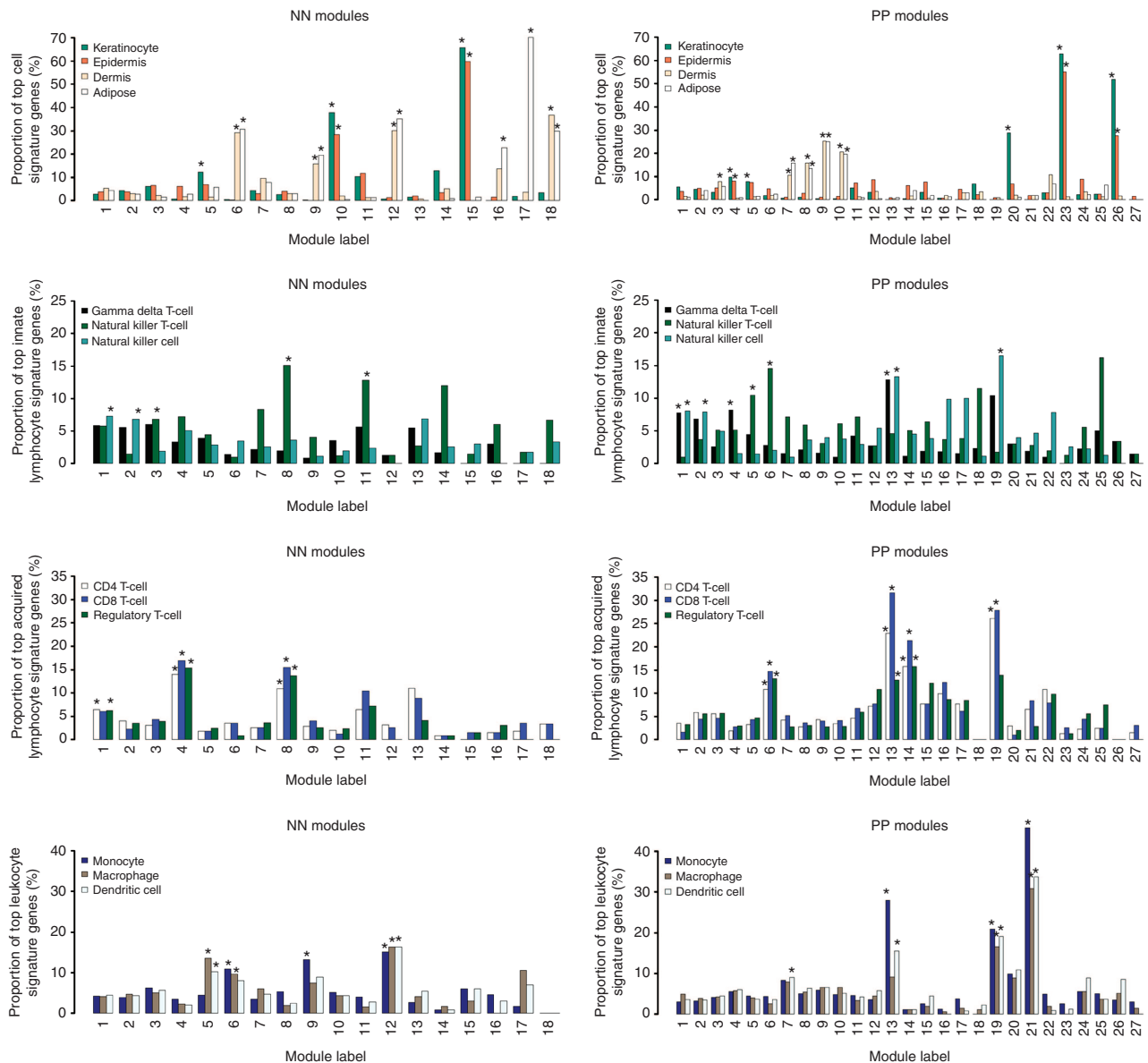


Figure 2. Proportion of top signature genes in different cell populations for coexpression gene modules constructed from normal (left panel) and psoriatic (right panel) skin samples. From top to bottom, respectively, the four panels show the proportions of signature genes for different skin cell and tissue compartments (keratinocytes, epidermis, dermis, and adipose tissue); “innate immunity” lymphocytes (gamma-delta T cells, natural killer (NK) cells, and NK-T cells); “adaptive immunity” lymphocytes (CD4+ T-cells, CD8+ T cells, and regulatory T-cells); and myeloid-derived leukocytes (monocytes, macrophages, and dendritic cells). Asterisks (*) denote significant (i.e., $P < 1 \times 10^{-4}$) enrichment for the top 5% of cell signature genes in the corresponding module.

and P26) were significantly enriched for signature genes induced by IL-17 treatment (Table 1). Module P20 was also enriched for signature genes upregulated by IL-1 α . This module was also significantly enriched for genes regulated by the transcription factors STAT1, STAT3, and interferon regulatory factor 3 (17 genes, $P = 8 \times 10^{-11}$; 11 genes, $P = 1.4 \times 10^{-9}$; and 14 genes, $P = 2.7 \times 10^{-9}$, respectively; Table 1 and data not shown).

IL37 (Nold *et al.*, 2012) was the most downregulated gene (FC = 0.10) in psoriatic module P23, which is strongly enriched for genes expressed in the stratum granulosum and regulated by *TINCR* and *STAU1* (Figure 3). We performed quantitative real-time reverse-transcriptase-PCR to measure *IL37* mRNA

and immunostaining to localize IL-37 protein with respect to the known keratinocyte differentiation marker loricrin. As shown in Figure 4, *IL37* mRNA was expressed at much lower levels in lesional psoriatic skin compared with nonlesional psoriatic skin and normal skin. IL-37 protein was readily detectable and confined to the stratum granulosum of normal and nonlesional psoriatic skin, where it colocalizes with loricrin, whereas it was undetectable in lesional psoriatic skin.

DISCUSSION

Although this is not the first study to apply RNA-seq to the study of psoriasis (Joyce *et al.*, 2011; Jabbari *et al.*, 2012), the availability of a much larger sample provides a more

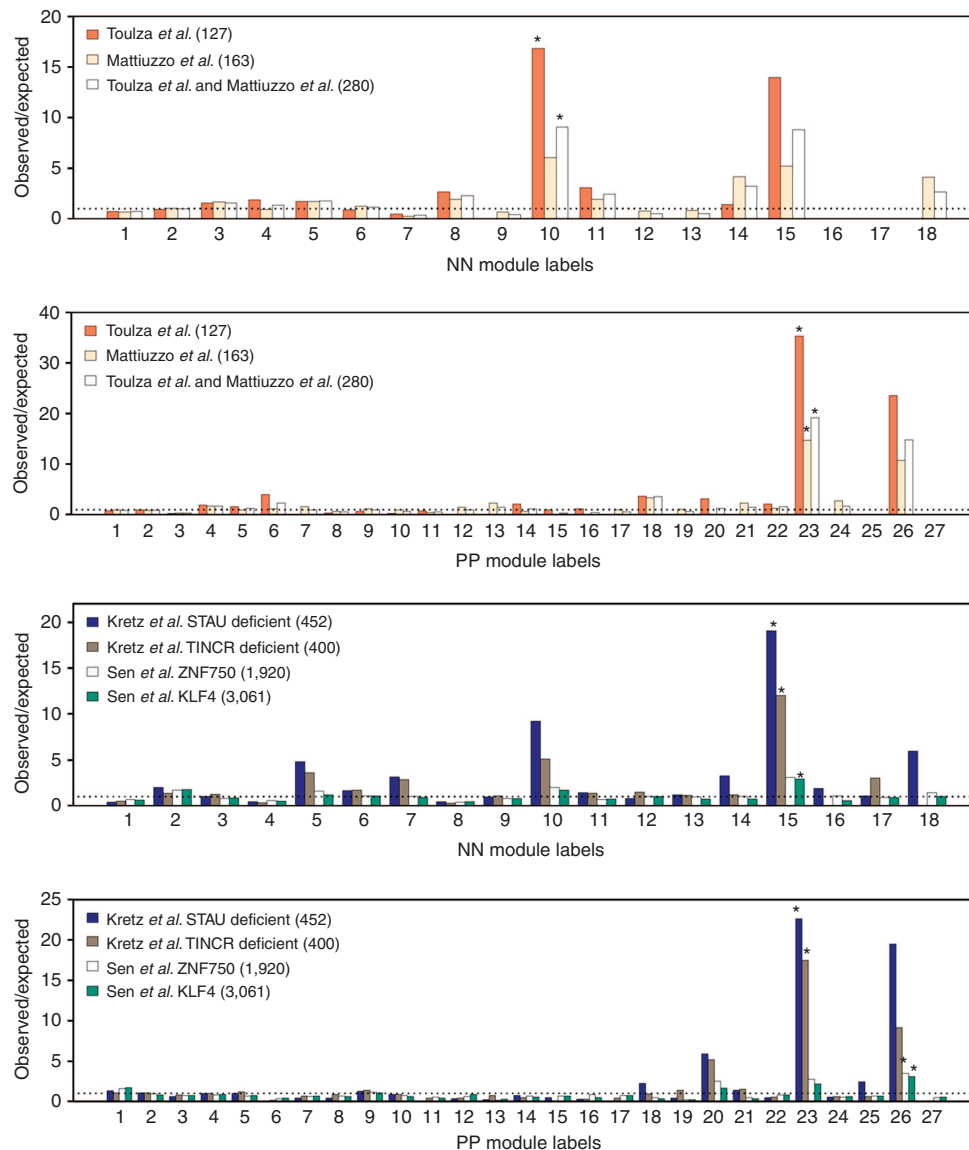


Figure 3. Overlap of gene coexpression modules with stratum granulosum and epidermal differentiation transcription factor target genes. The figures show the proportion of genes in each module overlapping with the genes identified in different independent studies. The top two panels depict overlap of each of the normal- and psoriasis-derived modules with stratum corneum genes identified by Toulza *et al.* (2007), Mattiuzzo *et al.* (2011), or the union of both studies. The bottom two panels depict overlap with targets of the epidermal differentiation regulators *STAU1* and *TINCR* (Kretz *et al.*, 2012) and with the transcription factors *ZNF50* and *KLF4* (Sen *et al.*, 2012). The numbers in the parentheses after the names of the studies indicate the number of genes in the corresponding gene sets used in this analysis.

comprehensive view of the processes influencing the psoriatic transcriptome. Many of the differentially expressed genes that we have detected by RNA-seq were not represented or detected in previous microarray studies on psoriasis (Zhou *et al.*, 2003; Reischl *et al.*, 2007; Yao *et al.*, 2008; Zaba *et al.*, 2009; Gudjonsson *et al.*, 2010; Suarez-Farinas *et al.*, 2010, 2012), including a recent meta-analysis (Tian *et al.*, 2012). Comparison of similarly powered experiments using the same statistical criteria revealed a substantial increase in the number of DEGs detected by RNA-seq, with little effect on the percentage of overlapping DEGs across studies (Supplementary Table S2 online).

Inspired by earlier observations of coordinate gene expression in psoriasis (Elder *et al.*, 1993; Kojima *et al.*, 1994; Hardas *et al.*, 1996; Elder and Zhao, 2002), a major feature of this study is the application of gene coexpression network analysis to the RNA-seq data. Although similar analyses have been fruitfully applied to the study of mouse skin carcinogenesis (Quigley *et al.*, 2009, 2011), to our knowledge this is its first application to human skin.

Links to immunological networks

WGCNA identified three coexpression modules in psoriatic skin that were significantly annotated for immune function

Table 1. Results of enrichment tests for the modules containing the highest proportions of signature genes in keratinocytes/epidermis

Condition	Module label	Hub genes ^b	Enrichment test ^a			
			Functions	Transcription factor	Gene signatures in cytokine-induced experiments	
					Up	Down
Normal	10	<i>TMEM54; PKP3; LYPD3; ST14; LAD1</i>	EDC; keratinization; 3' UTR-mediated translational regulation			
	15	<i>C1orf68; LY6G6C; LCE1B; NIPAL4; SLC46A2</i>	Keratinization		IL-17A (200 ng for 24 hours)	IL-1a (10 ng for 24 hours); IL-36g (5K ng for 24 hours); IL-22 (20 ng for 4 days)
Psoriatic	20	<i>SERPINB4; SERPINB3; SLC6A14; TGFA; HEPHL1</i>	Inflammatory response; immune response; extracellular space	<i>STAT1; STAT3; IRF3</i>	IL-17A (200 ng for 24 hours); IL-1a (10 ng for 24 hours); IL-1a (25 ng for 48 hours)	
	23	<i>LCE1C; LCE2B; LCE2C; LCE2D; C1orf68</i>	EDC; keratinization	<i>HR</i>		IL-36 g (5K ng for 24 hours); IL-36RA (5K ng for 24 hours); IL-36b (5K ng for 24 hours)
	26	<i>CNFN; TREX2; SPRR2B; SPRR2D; SULT2B1</i>	Keratinization; EDC; cornified envelope	<i>GLIS1</i>	IL-17A + TNF (10 ng for 24 hours); IL-17A + TNF (1 ng for 24 hours); TNF (1 ng for 24 hours)	IL-13 (20 ng for 24 hours)

Abbreviations: EDC, epidermal differentiation complex; IRF3, interferon regulatory factor 3; TNF, tumor necrosis factor; UTR, untranslated region. For the cytokine-induced experiments, the dosage (ng ml⁻¹) and treatment time are included in brackets.

^aOnly the top three most significant results are shown.

^bHub genes were defined as the genes having the highest similarity in the module, as assessed by the strength of correlation and the degree of shared neighbors based on the gene expression data, with other genes in the same module.

and enriched for signature genes of myeloid cells (P21), or both myeloid and T cells (P13 and P19; Figure 2). Consistent with the known inflammatory/immune cell infiltration characteristic of psoriasis lesions, each of these modules manifested increased proportions of upregulated genes in psoriatic skin (Figure 1, Supplementary Table S6 online).

Among modules significantly enriched for epidermis or keratinocyte signature genes, modules N15, P20, and P26 were enriched for transcripts induced by IL-17 treatment of keratinocytes *in vitro* (Table 1). Module P20 was also enriched for genes regulated by activation of STAT1, STAT3, and interferon regulatory factor 3 (Table 1). Together, these findings suggest that psoriatic keratinocytes are responding to a proinflammatory cytokine milieu in which IL-17 is upregulated (Supplementary Table S1 online) and biologically available (Di Meglio *et al.*, 2011; Zhang *et al.*, 2011; Shi *et al.*, 2012). This is of clinical relevance because biologicals targeting IL-17, its receptor, and both subunits of IL-23 are highly effective treatments for psoriasis (Lowes *et al.*, 2013), and IL-23 is a major signal supporting the expansion and survival of T cells expressing IL-17 (Kryczek *et al.*, 2008). As module P26 is highly upregulated in psoriasis lesions (Figure 1), keratinocyte differentiation-related (Figure 3) and IL-17 responsive (Table 1), it seems likely that IL-17 may

promote innate host defense in the skin by signaling to the outer layers comprising the epidermal barrier.

Links to epidermal lineage commitment

During development, lineage commitment occurs when multipotent stem cells are specified into three lineages—the epidermis, the hair follicle, and the sebaceous gland (Hsu *et al.*, 2011). In this study, we have identified gene coexpression modules that can be attributed to each lineage. Modules N10, N15, P23, and P26 were highly expressed in the epidermis (Figure 2). There was significant ($P < 1 \times 10^{-4}$) overlap between genes mapping to these modules and genes expressed in the stratum granulosum (Toulza *et al.*, 2007; Mattiuzzo *et al.*, 2011; Figure 3). *HOPX*, which encodes a homeodomain protein involved in regulating the balance between epidermal proliferation and differentiation (Obarzanek-Fojt *et al.*, 2010), maps to modules N15 and P23. *HOPX* is a target gene of the master epidermal development transcription factor p63 (*TP63*), as are several other downstream transcriptional regulators of epidermal differentiation including *GRHL3*, *KLF4*, and *ZNF750* (Sen *et al.*, 2012; Zarnegar *et al.*, 2012), all of which mapped to module N10. Notably, each of these genes maps to reported psoriasis susceptibility loci (Yang *et al.*, 2008; Birnbaum *et al.*,

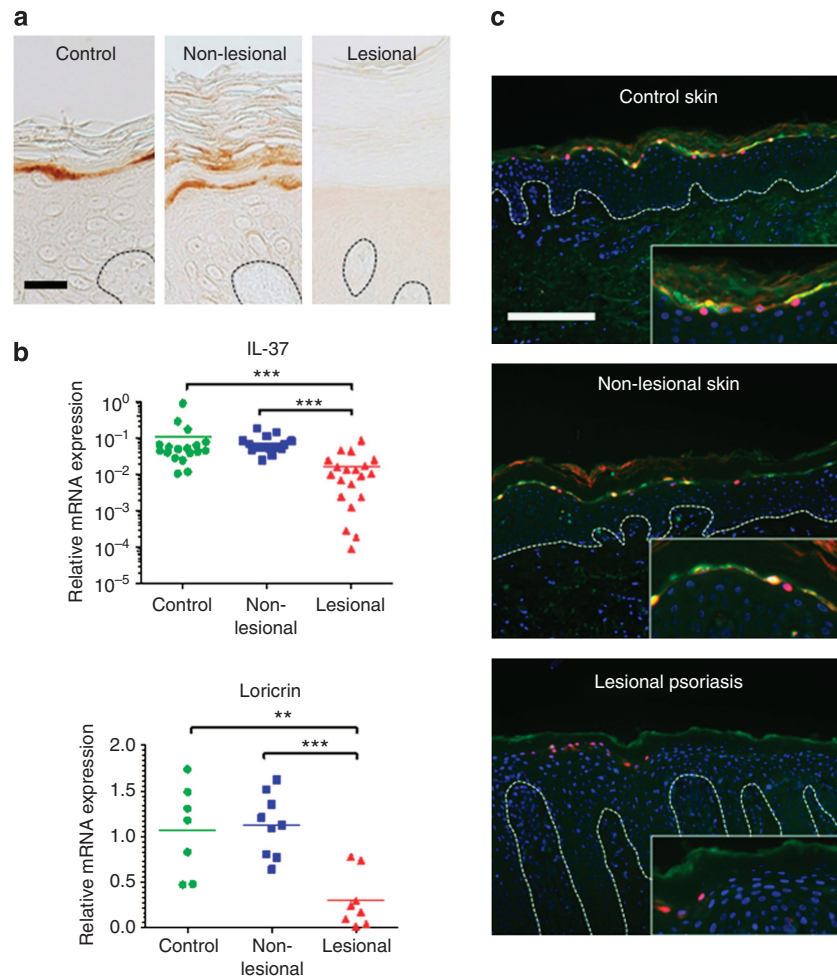


Figure 4. IL-37 is coexpressed with loricrin in the granular layer of nonlesional skin but significantly reduced in lesional psoriasis skin. (a) Immunohistochemistry revealed that although IL-37 protein (DAB, brown) is expressed in the granular layer of the epidermis of normal appearing skin, it is not detected in lesional psoriasis skin. Scale bar = 25 μm. (b) Quantitative real-time reverse-transcriptase-PCR (QRT-PCR) results showing downregulation of IL-37 and loricrin mRNAs in lesional psoriatic skin compared with nonlesional psoriatic skin. (c) Fluorescence immunohistochemistry showing that IL-37 (green) colocalized with loricrin (red) in the granular layer of nonlesional skin and this was undetectable in lesional skin. 4,6-Diamidino-2-phenylindole (DAPI) counterstaining of nuclei is shown in blue. Scale bar = 200 μm. Statistical significance as assessed by two-tailed *t*-test: ***P* < 0.01, ****P* < 0.001.

2011; Tsoi *et al.*, 2012). Modules N15 and P26 are significantly enriched ($P < 1 \times 10^{-4}$) with genes whose expression was altered by the knockdown of *KLF4* and/or *ZNF50* (Zarnegar *et al.*, 2012; Figure 3). We also found that modules N15 and P23 were significantly ($P < 1 \times 10^{-4}$) enriched with genes whose expression was altered by deficiency of the long noncoding RNA *TINCR* or the *STAU1* gene that is regulated by *TINCR*. Both of these genes are specifically involved in regulating the terminal phases of epidermal differentiation (Kretz *et al.*, 2012). The mapping of these transcription factors and their target genes to epidermal coexpression modules demonstrates that WGCNA is capable of detecting, *in vivo*, the activity of a p63-initiated and progressively focused transcription factor network that is engaged in the control of epidermal differentiation.

With regard to the hair follicle, the two modules most significantly enriched for function ("keratin filament", Supplementary Table S6 online), N14 and P18, were strongly

overlapping and contained many hair keratins (Schweizer *et al.*, 2007) and keratin-associated proteins that are specifically expressed in the hair follicle (Rogers *et al.*, 2006). Both clusters also contain *LHX2*, which encodes a hair follicle stem cell master transcription factor (Mardaryev *et al.*, 2011), and module N14 contains *MSX2*, another such factor. Both of these transcription factors, and most of the genes encoding hair keratins and keratin-associated proteins, have also been identified as part of a hair follicle gene coexpression network in mouse skin (Quigley and Balmain, 2009; Quigley *et al.*, 2011). This conclusion is further reinforced by the pronounced downregulation of many of the genes mapping to these modules in bald scalp (Garza *et al.*, 2012).

With regard to the sebaceous gland, module N17 was significantly enriched for signature genes expressed by adipose tissue (Plaisier *et al.*, 2009; Figure 2). We suspect that module N17 is reflective of sebaceous glands because all visible

subcutaneous fat was trimmed from our biopsies before analysis, and because this module was significantly enriched for PPAR-regulated genes (Supplementary Table S6 online). PPAR- γ stimulates sebocyte lipid production (Trivedi *et al.*, 2006) and protects sebocytes from apoptosis (Schuster *et al.*, 2011). Intimate anatomic and molecular relationships exist between the hair follicle, the sebaceous gland, and the arrector pili muscle (Lever and Schaumburg-Lever, 1990; Fujiwara *et al.*, 2011), and a clear connection has been observed between muscle and hair follicle-related gene coexpression networks in mouse skin (Quigley *et al.*, 2009). Consistent with these observations, we found that module N16 was significantly enriched for genes involved in muscle contraction (Supplementary Table S6 online).

Tissue architecture as a determinant of gene coexpression

Unlike cell cultures, tissue biopsies consist of multiple substructures comprising various cell types, which may differ considerably in normal versus pathological states (Swindell *et al.*, 2012, 2013). Upon annotation of the modules identified by WGCNA, we noticed that many of the downregulated modules were enriched for processes involving the extracellular matrix, which in skin is mainly found in the dermis. Noting that a much greater fraction of the cellular mass of a psoriatic biopsy is epidermal in origin than in a normal skin biopsy (Supplementary Figure S7e online), we reasoned that a larger fraction of the RNA in a psoriatic biopsy would be epidermal, and hence the dermal fraction would be lower. To test this, we identified two sets of genes from a data set obtained through laser-capture microdissection of epidermal versus appendage-free dermal tissue from normal versus uninvolved psoriatic skin (Mitsui *et al.*, 2012). These gene sets shared two properties, including (a) similar expression in laser-capture microdissection-dissected lesions and uninvolved skin, and (b) a trend toward dermis- or epidermis-specific expression. By querying our RNA-seq data set with the resulting two gene lists, we found an approximately 50% reduction in dermis-specific transcripts (Supplementary Figure S7 online). These results suggest that FC estimates for dermis-derived transcripts are driven downward by epidermal expansion in psoriasis lesions. This process is independent of intracellular shifts in gene transcription, but may nonetheless be a key factor influencing gene expression in full-thickness skin biopsies. Such changes would be predicted to involve not only dermis-derived mRNAs but also those from other cell types, including hair follicles, sweat glands, and immune/inflammatory cells, and indeed this is what we observe for hair follicle-derived clusters N14 and P18 (Supplementary Table S7 online). Our findings thus have general implications for transcriptome studies of inflammatory skin disease, or any context involving tissue samples that feature a complex mixture of minority and majority cell types.

In summary, these results bring unprecedented depth and resolution to the psoriatic transcriptome. They also illuminate an intricate gene coexpression network driven not only by inflammatory cells and transcription factors but also by disease-related differences in tissue architecture. Applied in tandem with histologically targeted approaches such as

microdissection-coupled transcript profiling, more detailed examination of coordinate gene expression offers great promise for an integrated understanding of the genetics, immunology, and cell biology of psoriasis in the coming years.

MATERIALS AND METHODS

All subjects involved in this study provided written informed consent under a protocol adherent to the Helsinki Guidelines and approved by the Institutional Review Board of the University of Michigan Medical School. Libraries for RNA-seq were generated from polyadenylated skin punch biopsy RNA and sequenced at one library per lane on the Illumina Genome Analyzer IIx. Reads were aligned to the reference genome NCBI build 37 using TopHat (Garber *et al.*, 2011), and expression was normalized to the number of reads per kilobase per million mapped reads. We used the Wilcoxon rank-sum test to identify DEGs. DEGs were detected based $P < 10^{-6}$ (corresponding to family-wise error rate < 0.025) with a FC > 2 . Functional annotation was performed using Gene Ontology (Ashburner *et al.*, 2000), KEGG (Kanehisa *et al.*, 2012), and Reactome (Matthews *et al.*, 2009). Transcription factor analyses were performed using the Ingenuity Pathway Analysis software (www.ingenuity.com). Significance thresholds were determined by Bonferroni correction. Coordinate gene expression analysis was performed using the WGCNA package (Horvath and Dong, 2008), with normal and lesional psoriatic skin samples being analyzed separately. Gene expression signatures were identified as described (Swindell *et al.*, 2012, 2013). Additional details are provided in the Supplementary Information online. The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE54456 (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54456>).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/jid>

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